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Antiproliferative Compounds of *Cyphostemma greveana* from a Madagascar Dry Forest^[1]

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Abstract

Bioassay-guided fractionation of the EtOH extracts obtained from a plant identified as *Cyphostemma greveana* Desc. (Vitaceae) led to the identification of one macrolide, lasiodiplodin (**1**), three sesquiterpenoids, 12-hydroxy-15-oxo-selina-4,11-diene (**2**), 1 β ,6 α -dihydroxyeudesm-4(15)-ene (**3**), and (7*R**)-opposit-4(15)-ene-1 β ,7-diol (**5**), and the new diterpenoid, 16,18-dihydroxykolavenic acid lactone (**4**). All the isolates were tested against the A2780 human ovarian cancer cell line, and compound **4** and a fraction containing **5** as the major constituent showed antiproliferative activities with IC₅₀ values of 0.44 μ M (0.14 μ g/mL) and 0.045 μ g/mL, respectively. A semisynthesis of compound **5** was carried out, but the pure synthetic compound was inactive, indicating that the activity of the fraction containing it must be due to a very minor and as yet unidentified substance.

Introduction

In our continuing search for bioactive molecules from the Madagascar forests as part of an International Cooperative Biodiversity Group (ICBG) program [2–5], we obtained an extract from the bark of the liana *Cyphostemma greveana* Desc. (Vitaceae). This extract, with an IC₅₀ value of 6.3 μ g/mL against the A2780 human ovarian cancer cell line, was selected for bioassay-guided fractionation based on this antiproliferative activity, and also on the absence of any previous phytochemical studies on this species. The only member of the genus *Cyphostemma* which has been previously studied phytochemically is *C. crotalarioides*, which has been shown to contain resveratrol-like antifungal compounds [6–8].

Bioassay-guided separation of the crude extract from the bark of *Cyphostemma greveana* furnished four known compounds and one new compound. The known compound **5** could

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only be obtained in partially purified form from a highly active but very minor subfraction, and its semisynthesis was thus carried out to test whether or not it was the active constituent of this fraction.

Results and Discussion

1. Isolation and Structure Elucidation

The crude extract of *C. greveana* bark (1.2 g) was subjected to liquid-liquid partition, guided by bioassay against the A2780 ovarian cancer cell line, which yielded a bioactive dichloromethane fraction (225 mg). Further fractionation by chromatography ultimately yielded the five compounds **1–5**. Compound **1** was identified as lasiodiplodin by comparison of its spectroscopic data with those of the known compound [9]. Compounds **2** and **3** were similarly identified as 12-hydroxy-15-oxo-selina-4,11-diene [10] and 1 β ,6 α -dihydroxyeudesm-4(15)-ene [11], respectively.

Compound **4** was obtained as a colorless powder. Its positive HRFABMS revealed a pseudo-molecular ion $[(M+H)^+]$ consistent with the molecular formula $C_{20}H_{30}O_3$, requiring six double-bond equivalents. The UV spectrum exhibited absorbance compatible with an α,β -unsaturated γ -lactone moiety (225 nm). Its 1H NMR spectrum showed the presence of two olefinic protons (δ_H 5.57, br s, H-3; 5.84, s, H-14), two OCH_2 groups (δ_H 4.73, br s, H₂-16; 4.09, br s, H₂-18), three methyls (δ_H 0.78, s, H₃-20; 0.81, d, J = 6.0 Hz, H₃-17; 1.08, s, H₃-19), and 14 protons in the region of 1.20–2.50 ppm (Table 1). Its ^{13}C NMR spectrum indicated the presence of one ester carbonyl group (δ_C 169.0, C-15), two double bonds (δ_C 168.8, C-13; 147.2, C-4; 120.9, C-3; 113.7, C-14), two oxygenated carbons (δ_C 73.7, C-16; 61.4, C-18), and thirteen signals in the range of 15.0 to 46.3 ppm (Table 1). The homonuclear 2D NMR COSY and TOCSY data for **4** identified connectivity sequences indicative of three coupling systems, H-3 through H₂-2 and H₂-1 to H-10 ($C=CH-CH_2-CH_2-CH$), H₂-6 through H₂-7 and H-8 to H₃-17 ($CH_2-CH_2-CH-CH_3$), and H₂-11 to H₂-12 (CH_2-CH_2). In the HMBC spectrum of **4**, the following correlations were observed: H₃-17 to C-7, C-8, and C-9; H₂-18 to C-3, C-4, and C-5; H₃-19 to C-4, C-5, C-6, and C-10; and H₃-20 to C-8, C-9, C-10, and C-11. These data indicated the structure of **4** excluding stereochemistry, and ROESY correlations between H₃-19 and H₃-20, H-8 and H-10 confirmed its relative configuration as that of 16,18-dihydroxykolavenic acid lactone. The acetate derivative of this compound has been reported previously [12], but this is the first report of the underlying natural alcohol.

The known compound (7*R**)-opposit-4(15)-ene-1 β ,7-diol (**5**) was identified as the major component in a very small partially purified fraction (0.8 mg) which had an IC_{50} value of 0.045 μ g/mL. Identification was made by comparison of the 1H NMR spectrum of the major

peaks with those published for the natural product [13], but it was not possible to deduce any structural information for the minor components of the mixture, nor was it possible to isolate a pure sample of compound **5**. Although it was deemed unlikely that **5** could have strong antiproliferative activity, a partial synthesis was carried out to determine whether or not **5** is the compound accounting for the antiproliferative activity against A2780.

2. Synthesis

The partial synthesis followed the published route (Scheme 1) [14] from germacrene D (**6**). Germacrene D was isolated from a sample of essential oil provided by the A. M. Todd Company, and it was epoxidized to epoxygermacrene D (**7**). Since only partial ^1H NMR data have been reported previously for this compound [15], complete assignments of the ^1H and ^{13}C NMR spectra of epoxygermacrene D were established using a combination of 1D and 2D NMR experiments, including COSY, HMQC, HMBC, and ROESY sequences. The relative configuration of **7** was confirmed by the large coupling constants of H-6 and H-7 ($J = 10.4$ Hz), H-9b ($J = 13.8$ Hz), and H-1 ($J = 11.0$ Hz), which indicated the axial orientations of those protons, and by the ROESY correlation of H-9b with H-1.

Acid catalyzed rearrangement of epoxygermacrene D (**7**) afforded a mixture of compounds, which were separated using normal and reverse-phase HPLC. The target compound, (7*R**)-opposit-4(15)-ene-1 β ,7-diol (**5**), was obtained with an isolated yield of 2.0%. The structure of the synthesized compound (**5**) was confirmed by comparing its spectroscopic data with those of the recorded data for this compound in the literature [13] and with the data for the natural product (**5**) from *Cyphostemma greveana*, and these three sets of data matched each other very well. In addition, the HPLC retention times of the semisynthetic and the natural compound were identical.

Several other sesquiterpenoid analogs were also obtained from the acid catalyzed rearrangement of **7**, and their structures were elucidated as **8–11** (isolated yields 10.8, 2.7, 0.9, and 10.6%, respectively). Their structures were elucidated using a combination of 1D and 2D NMR spectroscopy, mass spectrometry, comparison with the literature data, and chemical conversion; their ^{13}C NMR data are shown in Table 1. Compound **8** has been reported with unassigned stereochemistry at C-7 [14]. Compound **8** was thus hydrolyzed in 1 M NaOH in MeOH/H₂O (1:1) at 45 °C for 12 h to produce compound **5**, in which the configuration at C-7 must be *R** [13]. Thus, the relative configuration at C-7 of compound **8** was likewise determined as *R**. Compounds **9** and **11** were also reported along with compound **8**, but no complete NMR data were given for them [14]. In this study, complete assignments of their ^1H and ^{13}C NMR data are provided as shown in Table 1 and the Experimental Part. The known compound **10** belongs to the relatively rare class of isodaucane sesquiterpenoids, and its NMR data were identical to those in the literature [16,17]. Thus epoxygermacrene D was converted to a series of sesquiterpenoids with diverse skeletons.

3. Biological Activity

All the pure natural products (**1–4**), the fraction containing **5** and the synthetic compounds (**6–11**) were tested against the A2780 ovarian cancer cell line. Compounds **1** and **4** were active with IC₅₀ values of $5.8 \pm 0.7 \mu\text{M}$, $0.44 \pm 0.09 \mu\text{M}$, respectively, and the fraction containing **5** had an IC₅₀ value of $0.045 \mu\text{g/mL}$. Compounds **2**, **3**, and **6–11** were all inactive, with IC₅₀ values greater than $50 \mu\text{M}$. Lasiodiplodin (**1**) was previously isolated as a potent antileukemic macrolide from *Euphorbia splendens* [9]. The α,β -unsaturated γ -lactone moiety of compound **4** may be important for its activity against A2780 cells.

Regrettably the pure synthetic compound **5** was inactive, indicating that the activity of the fraction containing **5** must be due to a very minor and as yet unidentified substance. The lack of additional supplies of the original extract prevented further investigation of this substance.

Experimental Part

General Experimental Procedures

All reactions requiring anhydrous conditions were performed in oven-dried glassware under nitrogen or argon. Tetrahydrofuran (THF) was distilled over sodium/benzophenone, and dichloromethane (DCM) was distilled with calcium hydride. The other solvents were purchased in either HPLC or reagent grade. All reactions were monitored using analytical TLC plates (silica gel 60 GF, Merck, aluminum back), unless stated otherwise, and these TLC plates were visualized with either UV light at 254 nm or potassium permanganate (KMnO₄) solution or vanillin/sulfuric acid spray. Preparative TLC plates were either silica gel 60 GF, 1000 or 500 microns, 20 × 20 cm from *Analtech*, or aluminium oxide 60 F₂₅₄, 20 × 20 cm from *EM Science*, or C₁₈ silica gel, with 254 nm fluorescent indicator, 20 × 20 cm from *J. T. Baker*. Optical rotations were recorded on a JASCO P-2000 polarimeter. NMR spectra were obtained on Bruker Avance 600 equipped with a 1.7 mm CapProbe, JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. HRESI mass spectra were obtained on an Agilent 6220 TOF LC/MS. Chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. UV spectra were measured on a Shimadzu UV-1201 spectrophotometer. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative C₁₈ and phenyl Varian Dynamax column (5 μ m, 250 × 10 mm) and a preparative C₁₈ Varian Dynamax column (8 μ m, 250 × 21.4 mm). Finnigan LTQ LC/MS with a C₁₈ Hypersil column (5 μ m, 100 × 2.1 mm) was also used for crude sample analysis.

Antiproliferative Bioassays

Cytotoxicity measurements were performed at Virginia Polytechnic Institute and State University against the A2780 ovarian cancer cell line as previously described [18,19]. Paclitaxel (IC₅₀ 0.016 μ M) was used as the positive control. The A2780 cell line is a drug-sensitive ovarian cancer cell line.

Plant Material

Samples of the roots, bark, wood, and leaves of the climbing liana *Cyphostemma greveana* Desc. (Vitaceae) were collected on November 14, 2005 in the dry deciduous forest of Andranotsimaty, Vohemar, 10 km north-east of Daraina, Madagascar, at coordinates 13°11'04"S 049°42'24"E, and at an elevation of 182 m. The base of the trunk was bottle-shaped, and the liana had yellowish white flowers and green fruit. The voucher herbarium specimen was numbered as RIR 1308, and the plant was identified by Jeremie Razafitsalama (Missouri Botanical Garden).

Extraction Preparation

Dried bark of *Cyphostemma greveana* Desc. (250 g) was ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 3673 (3.54 g), of which 1.22 g was made available to Virginia Polytechnic Institute and State University.

Compound Isolation

Extract MG 3673 (1.05 g, IC₅₀ 6.3 μ g/mL) was suspended in aqueous MeOH (MeOH-H₂O, 9:1, 100 mL) and extracted with n-hexane (3 × 100 mL portions). The aqueous layer was

then diluted to 70% MeOH with H₂O and extracted with CH₂Cl₂ (3 × 100 mL portions). The CH₂Cl₂ extract (225 mg, IC₅₀ 2.2 µg/mL) was the most active, while the n-hexane extract (265 mg, IC₅₀ >20 µg/mL) was inactive and the aqueous MeOH extract (452 mg, IC₅₀ 8.2 µg/mL) was much less active than the CH₂Cl₂ extract. The CH₂Cl₂ extract (205 mg) was chromatographed on an open C₁₈ column (50 × 10 mm) using H₂O-MeOH (80:20 to 20:80, then 0:100) to yield the three fractions A [46 mg, IC₅₀ >20 µg/mL], B [127 mg, IC₅₀: 1.6 µg/mL], and C [23 mg, IC₅₀: 2.8 µg/mL]. Fraction B furnished four subfractions [subfraction I: 8.5 mg, IC₅₀: 1.1 µg/mL; subfraction II: 4.2 mg, IC₅₀: 0.16 µg/mL; subfraction III: 6.4 mg, IC₅₀: 0.33 µg/mL; and subfraction IV: 76.2 mg, IC₅₀: 2.5 µg/mL] after HPLC separation on a C₁₈ column (65% MeOH/H₂O). Subfraction III yielded compounds **1** (*t*_R 59 min, C₁₈, 65% MeOH/H₂O; 1.0 mg, IC₅₀ 1.7 µg/mL) and **2** (*t*_R 47.5 min, C₁₈, 65% MeOH/H₂O; 1.0 mg, IC₅₀ 0.14 µg/mL), while compound **3** (*t*_R 34.5 min, C₁₈, 65% MeOH/H₂O; 1.0 mg, IC₅₀ >2 µg/mL) was obtained from the less polar part of subfraction II. The two most active subfractions, II-7 (*t*_R 22 min, C₁₈, 65% MeOH/H₂O) and II-8 (*t*_R 23 min, C₁₈, 65% MeOH/H₂O) from subfraction II yielded compound **4** (*t*_R 10.5 min, C₈, 45% MeCN/H₂O; 0.4 mg, IC₅₀ >2 µg/mL) and fraction SC201-221-10 (*t*_R 13 min, C₈, 45% MeOH/H₂O; 0.3 mg, IC₅₀ 0.045 µg/mL), in which **5** was identified as the major compound (approximately 70%) by its NMR and MS data. An additional 0.6 mg of fraction SC201-221-10 was obtained from another 2 g of crude MG 3673 provided by Eisai Research Institute and Dow AgroSciences, but it was not possible to separate **5** from the other constituents or to identify any of the other constituents.

16,18-Dihydroxykolavenic acid lactone (4): colorless powder; [α]_D²⁵ -142° (*c* 0.14, MeOH); UV (MeOH) $\gamma_{\max}$ (log ϵ) 209 (3.83) nm; ¹H NMR (500 MHz, CD₃OD) δ _H 5.88 (1H, s, H-14), 5.54 (1H, s, H-3), 4.88 (2H, m, H-16), 4.03 (2H, m, H-18), 1.20-2.50 (14H, m), 1.11 (3H, s, H₃-19), 0.85 (3H, d, *J* = 6.4 Hz, H₃-17), 0.81 (3H, s, H₃-20); ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CD₃OD) see Table 1; HRFABMS *m/z* 341.2112 (MNa⁺; calcd for C₂₀H₃₀NaO₃, 341.2087).

(-)-Germacrene D (6)

A sample of a crude essential oil containing germacrene D (40% purity) was obtained from the *A.M.Todd* company. The crude oil (2.7 g) was subjected to column chromatograph over silica gel with elution by hexanes to yield 3 fractions. The most polar fraction consisted of (-)-germacrene D (0.64 g, 30%). [α]_D²⁵ -208.7° (*c* 1.0, CHCl₃). The ¹H and ¹³C NMR data were identical to the reported values for this compound [20,21].

Epoxygermacrene D (7)

NaHCO₃ (10% w/w in H₂O, 4 mL) was added in a well stirred solution of germacrene D (100 mg) in CH₂Cl₂ (2 mL), and the reaction mixture was cooled to 0 °C. *m*-Chloroperbenzoic acid (132 mg) in CH₂Cl₂ (4 mL) was slowly added dropwise to the reaction mixture. After 45 min, the reaction mixture was extracted thoroughly with aq. Na₂CO₃ (10% w/w, 5 × 10 mL). The organic layer was then washed with brine, dried over Na₂SO₄, and evaporated under reduced temperature to yield a colorless oil (104 mg), which consisted primarily of epoxygermacrene D (more than 85% purity judging from its NMR spectra). This sample was used directly in the next stage without further purification. ¹H NMR (500 MHz, CDCl₃): δ 6.04 (1H, d, *J* = 15.8 Hz, H-5), 5.48 (1H, dd, *J* = 15.8, 10.4 Hz, H-6), 4.92 (1H, br, s, H-11a), 4.90 (1H, br, s, H-11b), 2.58 (1H, br, d, *J* = 11.0 Hz, H-1), 2.50-2.54 (1H, m, H-3a), 2.25-2.31 (1H, m, H-3b), 2.17 (1H, ddd, *J* = 13.8, 6.0, 2.2 Hz, H-9a), 1.92-2.04 (1H, m, H-2a), 1.90-1.80 (2H, m, H-7 and H-8a), 1.52-1.38 (3H, m, H-8b, H-12, H-2b), 1.29 (3H, s, H₃-15), 1.03 (1H, td, *J* = 13.8, 1.95 Hz, H-9b), 0.90 (3H, d, *J* = 6.6 Hz, H₃-13), 0.85 (3H, d, *J* = 6.6 Hz, H₃-14). ¹³C NMR (500 MHz, CDCl₃): δ 148.3 (C-4),

136.6 (C-6), 133.1 (C-5), 112.6 (C-11), 69.0 (C-1), 61.4 (C-10), 51.9 (C-7), 38.9 (C-9), 32.5 (C-12), 31.2 (C-3), 30.2 (C-8), 29.2 (C-2), 20.9 (C-13), 19.7 (C-14), 16.6 (C-15).

Synthesis of (7R*)-opposit-4(15)-ene-1 β ,7-diol (**5**) and compounds **8** – **11**

Epoxystergermacrene **D** (83 mg) was dissolved in CH₂Cl₂ (2 mL), and aq. acetic acid (80% v/v, 2 mL) was added dropwise at 0 °C. After 1 h, the reaction mixture was adjusted to pH 9 by adding aq. Na₂CO₃. The organic layer was then washed by aq. Na₂CO₃ (10% w/w), H₂O, brine, dried (anhydrous Na₂SO₄), and evaporated under reduced pressure. The residue was separated by HPLC on a C18 column with elution by a step gradient of MeCN/H₂O (0–30 min, 60% MeCN; 30–35 min, 60–70% MeCN; 35–60 min, 70% MeCN). The peak with *t*_R 12.9 min was collected and evaporated, and the residue (~2 mg) was separated by HPLC on a silica gel column with elution by isopropanol/hexanes (1:10) to afford (7R*)-opposit-4(15)-ene-1 β ,7-diol **5** (*t*_R 14.4 min, 0.8 mg) and compound **10** (*t*_R 28.1 min, 0.3 mg). Collection of a peak at *t*_R 19.9 min and a second peak at *t*_R 21.9 min from the C₁₈ column afforded compounds **11** and **8**, respectively. A peak at *t*_R 19.3 min from the C₁₈ column was collected and subjected to HPLC on a silica gel column with elution by isopropanol/hexanes (1:10) to yield compounds **11** (*t*_R 10.9 min, 1.0 mg) and **9** (*t*_R 12.0 min, 1.1 mg).

(7R*)-opposit-4(15)-ene-1 β ,7-diol (**5**)

[α]_D²⁵ +103 (c 0.1, CHCl₃). The ¹H and ¹³C NMR data were identical to the reported values for this compound [13]. ¹H NMR (600 MHz, CDCl₃): δ 4.95 (1H, d, *J* = 1.6 Hz, H-15a), 4.80 (1H, d, *J* = 1.6 Hz, H-15b), 3.58 (1H, br, d, *J* = 10.7 Hz, H-1), 3.22 (1H, ddd, *J* = 9.7, 3.0 Hz, H-7), 2.28–2.35 (1H, m, H-6; 1H, m, H-3a), 2.08–2.14 (1H, m, H-3b), 1.80–1.95 (1H, m, H-8a; 1H, m, H-2a; 1H, m, H-5), 1.72–1.79 (1H, m, H-11; 1H, m, H-9a), 1.47–1.53 (1H, m, H-2b), 1.30–1.42 (1H, m, H-9b; 1H, m, H-8b), 0.99 (3H, d, *J* = 7.2 Hz, H₃-12/H₃-13), 0.90 (3H, d, *J* = 7.2 Hz, H₃-12/H₃-13), 0.66 (3H, s, H-14). ¹³C NMR (150 MHz, CDCl₃): δ 149.0 (C-4), 107.8 (C-15), 82.8 (C-7), 79.1 (C-1), 56.5 (C-5), 49.6 (C-10), 39.5 (C-6), 37.4 (C-9), 35.0 (C-3), 31.9 (C-2), 31.4 (C-11), 26.1 (C-8), 20.6 (C-13), 14.8 (C-12), 12.4 (C-14). HRESIMS *m/z*: 261.1826 [M+Na]⁺ (Calcd for C₁₅H₂₆NaO₂: 261.1830). A comparison of **5** with the major compound of active fraction SC201-221-10 described above (NMR, HPLC) confirmed that this major compound was identical to **5**.

(7R*)-opposit-4(15)-ene-7-acetoxy-1 β -ol (**8**)

[α]_D²⁵ +94.3 (c 0.4, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 4.85 (1H, d, *J* = 1.5 Hz, H-15a), 4.70 (1H, dd, *J* = 10.2, 2.4 Hz, H-7), 4.67 (1H, d, *J* = 1.5 Hz, H-15b), 3.53 (1H, ddd, *J* = 11.4, 4.9, 4.8 Hz, H-1), 2.40–2.46 (1H, m, H-6), 2.21 (1H, ddd, *J* = 13.6, 5.4, 2.0 Hz, H-3a), 1.92–2.00 (1H, m, H-3b), 1.91–1.87 (2H, m, H-11 and H-8a), 1.85 (1H, d, 10.5 Hz, H-5), 1.78 (1H, m, H-2a), 1.72 (1H, *J* = 10.5, 8.8 Hz, H-9a), 1.50–1.35 (4H, m, H-2b, H-8b, H-9b, and 1-OH), 0.93 (3H, d, *J* = 6.6 Hz, H-13), 0.87 (3H, *J* = 6.9 Hz, H-12), 0.62 (3H, s, H-14). ¹³C NMR (150 MHz, CDCl₃): see Table 1. HRESIMS *m/z*: 303.1940 [M+Na]⁺ (Calcd for C₁₇H₂₈NaO₃: 303.1931).

Sesquiterpene **9**

[α]_D²⁵ +159 (c 0.1, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 4.86 (1H, d, *J* = 1.5 Hz, H-15a), 4.58 (1H, d, *J* = 1.5 Hz, H-15b), 3.52 (1H, ddd, *J* = 11.3, 4.9, 4.9 Hz, H-1), 2.29 (1H, ddd, *J* = 17.4, 5.4, 2.0 Hz, H-3a), 2.13–2.19 (1H, m, H-6), 2.00–2.10 (1H, m, H-3b), 1.94 (1H, br, d, *J* = 13.6 Hz, H-7a), 1.79–1.83 (1H, m, H-2a), 1.68–1.74 (1H, m, H-9a), 1.62 (1H, dd, *J* = 14.2, 10.5 Hz, H-7b), 1.54 (1H, d, *J* = 12.3 Hz, H-5), 1.50–1.40 (2H, m, H-2b and H-9b), 1.49 (3H, s, H-13), 1.48 (3H, s, H-12), 1.37 (1H, d, *J* = 4.8 Hz, 1-OH), 0.65 (3H, s, H-14). ¹³C NMR (150 MHz, CDCl₃): see Table 1. HRESIMS *m/z*: 281.2117 [M+H]⁺ (Calcd for C₁₇H₂₉O₃: 281.2111); 303.1921 [M+Na]⁺ (Calcd for C₁₇H₂₈NaO₃: 303.1931).

Sesquiterpene 10

$[\alpha]_D^{25} +30.0$ (c 0.03, CHCl_3). ^1H NMR (600 MHz, CDCl_3): δ 5.39 (1H, d, $J=3.7$ Hz, H-5), 4.07 (2H, d, $J=5.7$ Hz, H-15), 3.49 (1H, ddd, $J=10.6, 5.4, 5.2$ Hz, H-1), 1.00 (3H, s, H-14), 0.92 (6H, t, $J=6.5$ Hz, H-13 and H-12), 2.30-1.20 (the rest of protons). ^{13}C NMR (150 MHz, CDCl_3): see Table 1.

Sesquiterpene 11

$[\alpha]_D^{25} +44.0$ (c 0.1, CHCl_3). ^1H NMR (600 MHz, CDCl_3): δ 5.11 (1H, t, $J=10.2$ Hz, H-6), 4.82 (1H, br, s, H-15a), 4.60 (1H, br, s, H-15b), 3.45 (1H, ddd, $J=10.9, 5.7, 5.2$ Hz, H-1), 2.30 (1H, ddd, $J=13.1, 4.9, 2.0$ Hz, H-3a), 2.06 (1H, br, dd, $J=13.2, 4.7$ Hz, H-3b), 1.98 (1H, d, $J=12.7$ Hz, H-5), 1.98 (1H, m, H-9a), 1.88 (1H, m, H-2a), 1.67 (1H, d of quintet, $J=7.0, 2.0$ Hz, H-11), 1.63-1.49 (2H, m, H-8a and H-2b), 1.43-1.25 (2H, m, H-8b and H-7), 1.35 (1H, d, $J=5.6$ Hz, 1-OH), 1.19 (1H, ddd, $J=13.2, 12.9, 3.7$ Hz, H-9b), 0.94 (3H, d, $J=7.0$ Hz, H-13), 0.89 (3H, d, $J=7.0$ Hz, H-12), 0.78 (3H, s, H-14). ^{13}C NMR (150 MHz, CDCl_3): see Table 1. HRESIMS m/z : 281.2122 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{17}\text{H}_{29}\text{O}_3$: 282.2111)

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Scheme 1.

Conversion of germacrene D to compounds **5**, **8** – **11**. a. *m*-chloro-peroxybenzoic acid, CH_2Cl_2 , 0°C ; b. 80% aq. AcOH, 0°C , 1 hr.

Table 1

NMR Data of Compounds 4, 5, 8 – 11

#	4	5	8	9	10	11
	¹ H ^{a,c}	¹³ C ^{b,e}	¹³ C ^{a,f}	¹³ C ^{a,f}	¹³ C ^{a,f}	¹³ C ^{a,f}
1	<i>d</i>	17.3	31.9	31.9	28.0	32.2
2	<i>d</i>	26.1	35.0	34.7	25.1	35.0
3	5.57 br s	120.9	149.0	146.3	138.1	144.5
4		147.2	56.5	55.8	131.0	53.5
5		37.5	39.5	38.2	51.4	70.8
6	<i>d</i>	36.0	82.8	82.1	55.8	49.0
7	<i>d</i>	27.0	26.1	25.9	27.1	18.1
8	<i>d</i>	36.2	37.4	37.0	39.7	36.1
9		38.5	49.6	49.4	46.3	42.2
10	<i>d</i>	46.3	31.4	30.3	33.0	26.2
11	<i>d</i>	35.2	14.8	15.4	20.0	16.0
12	<i>d</i>	21.8	20.6	20.2	22.2	21.3
13		168.8	12.4	12.2	20.0	11.6
14	5.84 s	113.7	107.8	107.5	67.4	107.8
15		169.0	79.1	79.0	76.7	79.2
16	4.73 br s	73.7				
17	0.81 d (6.0)	15.0				
18	4.09 br s	61.4				
19	1.08 s	20.3				
20	0.78 s	18.0				
CH ₃ C(O)O-			171.5	170.6		171.1
CH ₃ C(O)O-			21.2	22.7		21.2

^aIn CDCl₃^bIn CD₃OD.^cδ (ppm) 500 MHz; multiplicities; *J* values (Hz) in parentheses.^d1.20–2.50, 14H, m.

δ_e (ppm) 125 MHz. δ_f (ppm) 150 MHz.